In an effort to isolate the aro cluster-"ene (Gaertner and Cole 1977 Biochem. Biophys. Res. Commun. 75: 259) from *N. crassa*, we prepared a total BanHi digest of chromosomal DNA, ligated into the Saccharomyces shuttle vector pYE13 (Broach et al. 1979 Gene 8: 121), and amplified the resultant genomic library in *E. coli* (Procedure I). The amplified gene-bank was used to attempt complementation in four mutants of *E. coli* (see *E. coli* strains lacking aro activities by transformation (Procedure II)). Only the *E. coli* mutant SK288a (aroD), which lacks DHQase, was complemented. Isolation of plasmid DNA (Procedure III), and subsequent restriction of the DNA with BanHi, showed that the complementing activity was present on a 3.2 kbp BanHi fragment. This fragment in pYE13 also complements the qa-2 gene of *N. crassa* (Case and Giles, personal communication), but does not complement DHQase minus strains of *Saccharomyces cerevisiae* (Broach et al. 1979). We concluded that the 3.2 kbp BanHi fragment from *Neurospora crassa* carried the structural and promoter information of the qa-2 dehydroquinase gene.

Complementing activities for other *E. coli* arO mutations relevant to the aro cluster-gene have not been observed, suggesting either that BamHI cuts and inactivates these genes, or that some incompatibility exists between *E. coli* and *N. crassa* with respect to these genetic elements.

**Procedure I - Library Formation and Plasmid Amplification**

Ligation of 4.8 micrograms of BanHi digested *N. crassa* genomic DNA with 1.6 micrograms of BanHi digested, alkaline phosphatased, pYE13 DNA was done in a total volume of 800 microliters, also containing T4 DNA ligase buffer (lx T4 DNA ligase buffer: 50 mM Tris-C12 7.8, 10 mM MgCl2, 2 mM DTT, 1 mM ATP and 50 micrograms per ml of BSA) and 1600 U of T4 DNA ligase. The ligation was carried out for 18 h in a 15°C water bath. The 800 microliter ligation was then added to 16 ml of competent *E. coli* HB101 cells and our usual transformation was performed. The entire transformation mixture was added to 500 ml of LB-medium containing 25 micrograms per ml of ampicillin and grown to a stationary phase overnight in an air shake-incubator at 37°C. These cells were then spun down and resuspended in 10 ml of LB-medium plus 25 micrograms per ml of ampicillin. Enough of this suspension was added to 500 ml of M9 medium to bring the initial O.D. 600 to 0.16 using a 1 cm pathlength. The culture was then spun down in an air shake-incubator at 37°C until the O.D. 600 reached 0.8, at which time chloramphenicol was added to a final concentration of 200 micrograms per ml and the culture grown for an additional 18 h. The plasmid DNA was then recovered as described below in Procedure III.

**Procedure II - Bacterial Transformation**

Bacterial strains were made competent according to the following protocol. A single colony isolate was started in 20 ml of LB-medium and grown at 37°C overnight. The following day, a 560 microliter aliquot of the overnight culture was added to 56 ml of LB-medium in a 250 ml side-arm flask with a 1 cm pathlength. The culture was then grown to an O.D. 600 of 0.16 and the flask placed in ice-water for 10 minutes. The cells were then spun down in sterile 30 ml oak-ridge tubes, resuspended in 28 ml of C.M.T. buffer (lx C.M.T. buffer: 0.1 M CaCl2, 5 mM MgCl2, 10 mM Tris-C12 7.8), and incubated on ice for 25 minutes. The cells were then spun down again and resuspended in 1.4 ml of C.M.T. The transformation procedure was begun by the addition of DNA to the competent cells in a ratio of 5-10 micrograms of aqueous DNA per 200 micrograms of competent cells, and incubated on ice for 15 minutes. After a 5 minute heat shock in a 37°C water-bath, 800 micrograms of LB-medium was added per 200 microliter transformation aliquot, and the culture vigorously shook in a 37°C water-bath for 60 minutes. The transformation mixture was then plated on selectable medium or amplified directly.

**Procedure III - Plasmid Purification**

Plasmid purification was done according to the alkaline lysis protocol in the Cold Spring Harbor laboratory manual "Molecular Cloning," T. Maniatis, E.F. Fritsch and J. Sambrook (1982), without modification.

<table>
<thead>
<tr>
<th>LB-Medium (1000 ml)</th>
<th>M9 Medium (1000 ml)</th>
<th>10X M9 Salts (500 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 g tryptone 100 ml</td>
<td>10X Mg salts (see below)</td>
<td></td>
</tr>
<tr>
<td>5 g yeast extract</td>
<td>5 g casamino acids</td>
<td></td>
</tr>
<tr>
<td>5 g NaCl</td>
<td>15.0 g Na2HPO4, 7H2O</td>
<td></td>
</tr>
<tr>
<td>0.5 ml 5 N NaOH</td>
<td>54.7 g Na2HPO4, 7H2O</td>
<td></td>
</tr>
</tbody>
</table>

**E coli Strains**

The "SK" strains of *E. coli* were a generous gift from Si dnev R. Kushner (The University of Georgia, Athens).

SK494: F' gal- thi- araE2, spcR, his-4, endA, sbaB15, (DHs reductase)
SK3337: F', gal-, K2, avcl, H1, xyl-, his-4, pro-A, thi-1, tetR, λ− araO2, hsdR4, (ESP synthase)

Hbl Cl: hsdR, hsdM, recA, supE44, lac2, leuB6, proA2, thi-1, SinR

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